THE HECHT-WEINBERG-GRADWOHL TEST

STUDIES ON THE SERODIAGNOSIS OF SYPHILIS I.

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The discovery of the "complement-fixation reaction" (Bordet-Gengou phenomenon) by Bordet,[1] opened a new epoch in the serodiagnosis of the infectious diseases, and offered as well a means by which specific antibodies could be demonstrated. Wassermann and his co-workers,[2] shortly after the announcement of Bordet's discovery, applied the principle of complement fixation to the serodiagnosis of syphilis. They assumed that patients suffering from this infection carry specific antibodies in their blood, which, when brought in contact with the specific antigen, form a complex that absorbs or fixes complement. Their efforts were rewarded by most encouraging results, and stimulated other workers to make investigations along similar lines. It was soon demonstrated that the socalled syphilitic antigen need not contain spirochetal substance, but that certain tissue extracts (lipoidal in character) filled the antigenic requirements fully as well as the Wassermann antigen prepared from tissue rich in spirochetes. This proved that the reaction does not depend on a specific antigen in the true sense of the term, and the interesting question arose whether the bodies in the serum of syphilitic persons were true antibodies against spirochaeta pallida or other substances, possibly metabolites, produced in the body through the ravages of the disease. Probably the latter view has more evidence in its favor, although the question is far from being settled. Whatever may be the nature of the substances in the serum of syphilitic persons which enter the reaction, we know that they are not particularly thermostabile, and in the process of "inactivating" such serums by heating even at 56 C. quite a decided destruction of the "fixing bodies" occurs (Noguchi [3]). To overcome this particular objection to the original Wassermann technic modifications of the method have been suggested. Among these that of Hecht and Weinberg [4] has perhaps received the most attention, especially a modifi-

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[1] Ann. de l'Inst. Pasteur, 1900, 14, p. 257; 1901, 15, p. 290.
[2] Deutsch. med. Wchnschr., 1906, 32, p. 745.
[3] Serum Diagnosis of Syphilis, 1912, p. 96.
[4] Wien. klin. Wchnschr., 1909, 22, p. 256.

cation suggested by Gradwohl.[5] Gradwohl, by his modification, claims that a positive reaction may be obtained with the serum of syphilitic persons in a larger number of cases (15% or higher) than by the usual Wassermann technic, which he employed. His claims are supported by certain other workers (Blaivas,[6] Christian[7]).

Most serologists have occasionally observed that the serum from certain syphilitic persons fails to give positive reactions by the former test method. Any practical modification which will give positive reactions in such cases is quite worthy of investigation and adoption if its superior merits are proved. On the other hand, the tendency of certain serologists to employ oversensitive tests which may give pseudo-positive reactions is a dangerous procedure, and one which should be discouraged. It is quite difficult to delimit the borderline between the nonsyphilitic and the syphilitic person. Supersensitive laboratory tests on nonsyphilitic serums in certain cases may give results which pass over to the syphilitic side. This is especially true when the worker is in pursuit of those who clearly show syphilitic conditions, but whose serums fail to give a positive reaction with the usual methods. The nonreacting syphilitic may not even carry "syphilitic fixing bodies" in his serum. It is well known that there is a decided quantitative difference existing between the different positive serums. If these "fixing bodies" are metabolites, they may arise anew, or may be normal substance markedly increased in amount during the course of the disease. In the latter case, it is quite conceivable that certain nonsyphilitic persons may naturally have an abnormally large content of these substances in their blood, while, on the contrary, known syphilitic persons may fail to elaborate these bodies and show only a very low content, even below the average norm for the healthy person. Therefore members of either group may be found on opposite sides of an arbitrarily established borderline, where clinically they do not belong. Such possibilities must be considered, and caution must be exercised by the serologist not to place the stigma of syphilis on the noninfected by applying a test which picks up the occasional elusive syphilitic serum, but also gives a positive reaction in certain nonsyphilitic persons.

As to the criteria which should determine the syphilitic from the nonsyphilitic persons, no common agreement exists. By cooperative studies among clinicians, pathologists and serologists, progress may

[5] Jour. Am. Med. Assn., 1914, 53, p. 240; 1917, 68, p. 514. Am. Jour. Syph., 1917, 1, p. 450.

[6] Jour. Lab. and Clin. Med., 1919, 5, p. 244.

[7] Am. Jour. Syph., 1919, 3, p. 613.

be made toward that ultimate end. In the absence of a standard method for the serum diagnosis of syphilis, a multitude of modifications of the original Wassermann technic have come into existence. Many of these modifications are erroneous in conception, even conflicting with the established laws of serology, consequently leading to false results. Owing to the fact that no absolute criterion exists by which the presence or absence of syphilis may surely be determined in a person, the acceptance of any particular laboratory test as an exact basis to evaluate another method must be dependent on a most critical analysis. The basic method applied in such studies may be faulty, and give rise to misleading conclusions. Recognizing these conditions, the investigator should use as a basis a method which experience has shown gives trustworthy results as a diagnostic means.

In undertaking the present study, the authors have attempted to determine the comparative serodiagnostic relationship existing between a modified Wassermann technic,[8] which has been found quite satisfactory, and the Hecht-Weinberg-Gradwohl technic, when applied to the serum of a group of general hospital cases. In carrying out the latter technic, utmost care was exercised to conform with the Gradwohl test as published. The radical difference between the two tests lies in the source of the complement and the antisheep immune body. In the modified Wassermann technic employed, the serums were inactivated by heating in the water bath for 30 minutes at 54 to 56 C. to destroy the natural complement present. An accurately standardized amount of complement was then added in the test, and finally, a constant amount of sensitized sheep corpuscles, as an indicator for the degree of complement fixation. In the Hecht-Weinberg-Gradwohl modification, fresh active serum of the patient is used, advantage being taken of the presence of complement and natural antisheep hemolytic immune body (amboceptor) which most persons possess. When both complement and hemolytic immune body are present the compensating phenomenon of von Dungern [9] and of Morgenroth and Sachs [10] is applied to determine the amount of serum which will just lake a given amount of the red blood cells of the sheep. This is called the "hemolytic index," and is expressed numerically, depending on the amount of 5% suspension of sheep blood corpuscles completely laked by 0.10 c c of the fresh human serum under specified conditions. If

[8] Wood, Vogel and Famulener: Laboratory Technique, 1922, p. 247.
[9] München. med. Wchnschr., 1900, 47, p. 677.
[10] Berl. klin. Wchnschr., 1902, 39, p. 817.

the given amount of test serum is found to lake 0.10 c c, or its multiple of sheep corpuscle suspension, the index is indicated by the corresponding number, i. e., 0.10 c c gives a "hemolytic index" of 1 . . . 0.4 c c of 4; etc. In case the hemolytic index is low, that is, under 4, the test is omitted as without value over the Wassermann method, and those serums which show no hemolysis are not applicable to the test. In each method the same antigen (acetone-insoluble antigen of Noguchi) was used, and in addition with the Wassermann method a cholesterinized antigen was used.

Two series of tests were carried out on serums submitted to the laboratory for routine tests. All were used in the fresh condition, some only shortly after the blood was taken, none after 24 hours. These were kept in the icebox when not tested immediately after drawing blood.

In the first series of 50 serums, only 7 were found to possess sufficient hemolytic power for the advantageous application of the test. Of these, only one gave positive results with both methods; one gave a questionable positive with the Hecht-Weinberg-Gradwohl method and negative with the Wassermann method with the acetone-insoluble antigen, but a low positive with the cholesterinized antigen. The history of this case might be considered suspicious of syphilis, although no definite clinical evidence was shown. The remaining 5 serums gave negative results with both methods. Since the sheep corpuscles used in this test were from an old laboratory animal, which had been used for bleeding purposes over a considerable time, it was thought that perhaps its cells had become resistant to hemolysis by the human system employed. This possibility might account for the relatively small percentage of human serum found suitable for test in the foregoing series.

In a similar manner a second series of tests on 100 serums was undertaken, using the blood from a sheep which previously had been bled little. Of these, only 34 were found suitable (hemolytic index 4 or higher) for testing. Twenty-nine of this group gave negative reactions, both with the Hecht-Weinberg-Gradwohl and the Wassermann method when the acetone-insoluble antigen was used, but one of these gave a positive (+ + + +) reaction by the latter method with the cholesterinized antigen. Of the 5 which gave positive reactions with the Hecht-Weinberg-Gradwohl test, one serum (hemolytic index, 5) which was weakly positive, was negative in the Wassermann test with the acetone-insoluble antigen, but gave a doubtful reaction (±) with the

cholesterinized antigen—this was a treated case of syphilis. The serum which was negative with the acetone-insoluble antigen by both methods, but strongly positive (+ + + +) with the cholesterinized antigen by the Wassermann method, had a hemolytic index of 4. This patient gave a history of having contracted syphilis 10 years before and had undergone hospital treatment, including treatment with arsphenamin. In this series, 6 patients had a syphilitic history. If a serum were negative by the Wassermann technic with the cholesterinized antigen, in all probability the patient was nonsyphilitic or at most "cured." Since this test with this antigen was considered as highly sensitive, it was used as a means of excluding nonsyphilitic persons.

Gradwohl states that 98% of patients have sufficient natural antisheep amboceptor and complement to carry out the test, while Christian [7] found only 93.3% in his series. Wade,[11] in his studies on natural hemolysins in fresh human serum, found that the antisheep was absent in 16%, and that it showed great variability in concentration. In our small series we found that considerable discrepancies existed in the results when tested with the red blood cells of 2 different sheep. As the same serums were not tested in parallel with corpuscles from the 2 animals, it is not possible to say whether this was a coincidence or due to greater resistance of the erythrocytes of one of the animals. We suspect the latter. If a hemolytic index of 1 be accepted as a basis for test purposes, our results would show that 22% of the first and 10% of the second series would be unsuitable. But since Gradwohl states that his modification has no advantage over the Wassermann technic except in serums with a hemolytic index of 4 or over, we find that only 14% in the first and 34% in the second series fall into that category. As is evident, our results indicate that only a relatively small percentage of serums was applicable where we might expect the greatest value of the test. Also, since the relative amounts of antisheep immune body and complement in the serum of different persons are exceedingly variable, the accuracy of the complement fixation test may be questionable in some instances. This source of error has long been recognized by many serologists, and has been pointed out in particular by Ottenberg.[12]

SUMMARY

The number of serums (150) examined in the 2 series of tests herewith reported is quite inadequate for purposes of definite conclusions.

[11] Jour. Med. Research, 1916, 29, p. 113.
[12] Jour. Immunol., 1917, 2, p. 39.

However, we found that the reactions with the Hecht-Weinberg-Gradwohl test were hardly so marked as with the Wassermann technic, but the Gradwohl modification might be considered somewhat superior when relying only on the results with the acetone-insoluble antigen in each method. On the other hand, the use of the cholesterinized antigen with the Wassermann method showed a stronger reaction with each positive serum (with one exception), and further gave a strongly positive reaction in one case—tertiary syphilis—which was negative with the Hecht-Weinberg-Gradwohl technic. In this report all serums giving positive reactions came from patients who gave a clear clinical history of syphilis, with the exception of one who had a suspicious history.